

Section II (Remarks)**A. Summary of Amendment to the Claims**

By the present Amendment, claim 1 has been amended and claims 2 and 3 have been cancelled. No new matter within the meaning of 35 U.S.C. §132(a) has been introduced by the foregoing amendments. Specifically, support for the amendments to claim 1 is provided by claims 2 and 3, as originally filed.

Thus, upon entry of the amendments, claims 1 and 4-10 will be pending, of which claims 8 and 10 are withdrawn.

B. Information Disclosure Statement

The examiner noted that a copy of the cited Bradford reference was not included with the IDS filed July 1, 2005. A full text copy of this reference is attached hereto as Exhibit A. Substantive consideration of the reference is respectfully requested.

C. Rejection of the Claims Under 35 U.S.C. §112

Claims 1-7 and 9 were rejected in the Office Action mailed January 21, 2009 as vague and indefinite. Specifically, the examiner alleged that claim 1 lacks a transitional phrase between the preamble and the body of the claim and therefore fails to define the metes and bounds of the claimed subject matter.

The examiner's attention is respectfully drawn to claim 1, as amended above, where the term "comprising" has been added to claim 1. Furthermore, a "wherein" clause has been added to the claim in order to clarify the structure of the protein chip. Support for the substance of the wherein clause is in original claims 2 and 3.

As amended, the claim meets the definiteness requirements of 35 U.S.C. §112, second paragraph. Withdrawal of the rejection is respectfully requested.

D. Rejection of the Claims Under 35 U.S.C. §102

In the Office Action mailed January 21, 2009 the examiner rejected claims 1, 6, 7 and 9 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent Application Publication No. 2002/0028463 A1 (hereinafter "Duffy"). Applicants respectfully disagree.

Anticipation of a claim requires the disclosure in a single prior art reference of each element of the claim under consideration. (Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).)

By the present Amendment, claim 1 has been amended to read as follows:

A protein chip of a S-L-SP form comprising a solid substrate (S) and a substrate peptide (SP) immobilized on the solid substrate (S) by a linker protein (L) of leptin or malic enzyme, wherein the substrate peptide is fused with the linker protein in the form of a peptide monomer, a dimer of monomer-proline-monomer, or a multimer where monomers are linked to each other by a proline.

Specifically the examiner's attention is drawn to the recited elements "...a linker protein (L) of leptin or malic enzyme..." and "...wherein the substrate peptide is fused with the linker protein in the form of a peptide monomer, a dimer of monomer-proline-monomer, or a multimer where monomers are linked to each other by a proline..." The subject of such claim elements was previously contained in dependent claims 2 and 3, not included in the rejection of the claims over Duffy.

Duffy does not describe a linker of leptin or malic enzyme. The examiner cited to page 10 of Duffy, para. [0091] as describing a "linker protein," as claimed. Applicants respectfully disagree. The linker described by Duffy and exemplified in Fig. 4, also cited by the examiner is provided as a linker between a biomolecule and a SFM. In Duffy, "SFM," as defined on page 6, para. [0052], is used to refer to SAM-forming molecules (where SAM stands for "self-assembling monolayer"). SFMs are formed on a surface and serve as "intermediary molecules which bind biomolecules and which are immobilized on or form a layer on the surface." The linkers cited by the examiner link biomolecules to SFMs, which are further bound to the surface substrate. The linkers (L) do not link a substrate peptide (SP) directly to a solid substrate (S), as recited in claim 1, in the form "S-L-SP." Additionally, nowhere in Duffy are linkers of leptin or malic enzyme described.

Since Duffy does not describe the linker element of the claim, Duffy does not anticipate claim 1. Claims 6, 7 and 9, while method claims, recite a method using the chip of claim 1. As claims dependent from claim 1, claims 6, 7 and 9 contain all elements of claim 1. Since claim 1 is not anticipated by Duffy, claims 6, 7 and 9 are also not anticipated by Duffy.

Since Duffy does not describe a protein chip or method as set forth in claims 1, 6, 7 and 9, Duffy does not anticipate the claimed invention. Accordingly, withdrawal of the rejection of claims 1, 6, 7 and 9 under 35 U.S.C. § 102(b) as being anticipated by Duffy is respectfully requested.

E. Rejection of the Claims Under 35 U.S.C. §103

In the Office Action mailed January 21, 2009 the examiner rejected claims 1-6 as obvious under 35 U.S.C. § 103(a), over MacBeath et al., *Science*, Sept. 8, 2000, vol. 289, pp. 1760-3 (hereinafter “MacBeath”) in view of U.S. Patent No. 6,335,176 (hereinafter “Inglese”). Applicants respectfully disagree.

By the present Amendment, claim 1 has been amended and claims 2 and 3 have been cancelled. Accordingly the rejection under 35 U.S.C. § 103(a) is addressed herein as applicable to pending independent claim 1 and claims 4-6 dependent therefrom.

In the wake of the recent U.S. Supreme Court decision in *KSR International Co. v. Teleflex Inc.*, No. 04-1350, 550 U.S. ____ (April 30, 2007) MPEP §2143 provides examples of rationales to support a conclusion of obviousness. Such exemplary rationales that may support a conclusion of obviousness include:

(A) Combining prior art elements according to known methods to yield predictable results...[and]

(G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

By the present rejection it appears that the examiner based the rejection on the assertion that the result of the combination of the array and method elements of MacBeath with the reagents and compounds of Inglese by known methods would have been predictable to one of skill in the art. Applicants respectfully disagree. As detailed in MPEP §2143, in order to reject a claim based on any of the above-identified rationales, the examiner must demonstrate predictability of the results. Specifically, under Rationale A, the examiner must demonstrate “a finding that one of ordinary skill in the art would have recognized that the results of the combination were predictable.” Under each exemplary rationale listed in MPEP §2143, it is provided that “[i]f any of these findings cannot be made, then this rationale cannot be used to support a conclusion that the claim would have been obvious to one of ordinary skill in the art.” Applicants respectfully

assert that one of skill in the art could not have predictably arrived at the presently claimed invention from the combination of MacBeath in view of Inglese, as cited by the examiner, and that there would have been no logical reason for one of skill in the art to combine such references.

By the present Amendment, claim 1 has been amended to include the subject matter previously recited in dependent claims 2 and 3. Claim 1 and claims 4-6, dependent therefrom, are not obvious in view of the cited references.

MacBeath is provide by the examiner as “teach[ing] protein microarrays...for high throughput function determination...A variety of chemically derivatized slides...can be printed[,] for example slides treated with aldehyde-containing silane reagent. These aldehydes can react readily with primary amines on the proteins...” (Office Action mailed January 21, 2009, p. 7.) The examiner further asserts that MacBeath “teaches a method for analyzing interaction between a reactive protein and the substrate peptide...” as claimed in claim 6. Applicants respectfully disagree.

As acknowledged by the examiner, MacBeath is silent on teaching that the substrate peptide is immobilized on the solid substrate by the mediation of a linker protein. (Office Action mailed January 21, 2009, p. 7.) As amended, claim 1 recites a particular linker that is leptin or malic enzyme. MacBeath does not provide a substrate peptide so attached by a linker to a solid substrate.

The examiner cited Inglese in combination with MacBeath, as providing a linker, such that the combination of MacBeath and Inglese renders the claimed invention obvious. Applicants respectfully disagree.

Inglese does not teach **a fusion protein** with a substrate **protein** fused to a linker **protein**. In Inglese the reagent is described with structure A-B-C, where A is a moiety that is specifically reactive with a reactive side chain, B is a linking moiety, and C is a peptide sequence that contains a kinase substrate (Abstract). The function of such reagent is “incorporating phosphorylation sites into compounds.” An intermediate compound is formed after reaction of the reagent with a starting compound, where the intermediate compound is a phosphorylatable compound that can be phosphorylated by reaction with kinase specific for element C of the

reagent (col. 2, ll. 20-23). The resulting compound is therefore a phosphorylated version of the starting compound (col. 9, ll. 7-11).

None of the above described compounds, the reagent, the starting compound or the resulting phosphorylated compound, is similar to the S-L-SP element of claim 1 of the present application. As claimed, the S-L-SP is a fusion protein where the **substrate peptide is fused with the linker protein** (leptin or malic enzyme) in the form of a peptide monomer, a dimer of monomer-proline-monomer, or a multimer where monomers are linked to each other by a proline.

By contrast, the starting compound (protein) of Inglese is merely linked to a **substituted or unsubstituted succinimidyl moiety** by reaction of the “A” moiety of the reagent (A-B-C) with an amine group or thiol group of the protein (claim 1; col. 7, ll. 53-55). Where leptin is included, as cited by the examiner, it is in generation of a leptin-peptide A conjugate and a ³³P labeled leptin-peptide A conjugate. Contrary to the examiner’s assertion, the resulting compounds of Inglese are merely target proteins for analyzing, not a linker protein which can link a solid substrate with a substrate peptide.

Additionally, the reagent of Inglese contains elements A-B-C. The **“A” moiety** is merely a moiety that is **specifically reactive with a reactive side chain** such as an N-hydroxysuccinimide ester (claim 6; col. 3-4). The **“B” moiety** is the **reactive chemical material** such as BMH (bismaleimido-hexane), 1,4-di[3-(2-pyridyldithio)-propionamido]]butane, and so on (claims 1-3, 6 and 8; col. 4). The “B” moiety is not a protein such as malic enzyme or leptin protein.

Accordingly, the disclosure of Inglese does not remedy the deficiencies of MacBeath. The combination of MacBeath in view of Inglese still fails to provide a showing of a substrate peptide immobilized on a solid substrate by the mediation of a linker protein.

Further, one of skill in the art would not have had motivation to combine the MacBeath and Inglese references. It is an object of the present invention to allow an increase in the reactivity between a peptide with low molecular weight and an enzyme with the high molecular weight and between the peptide and the reactive antibody.

By contrast, the object of Inglese is to provide a method to chemically modify already existing proteins and peptides so that they become substrates for protein kinase phosphorylation. Namely, Inglese teaches a method that allows introduction of phosphorylation site in a protein in

order to label the protein with ^{32}P or ^{33}P (claims 14, 15; col. 9, ll. 60-65). In Inglese, the peptide sequence containing a kinase substrate merely provides a phosphorylation site for labeling, not to allow an increase in the reactivity between a peptide with low molecular weight and an enzyme with high molecular weight.

The examiner's attention is respectfully drawn to the examples of the present invention. When only the substrate peptide such as the low molecular weight kemptide was immobilized on the protein chip, its interaction with the antibody did not occur, but when the peptide in a form fused with the linker protein such as leptin or malic enzyme was immobilized, specific interaction with the antibody did occur. No such interaction would be expected from an array resulting from the combination of MacBeath and Inglese.

One of skill in the art would not have been motivated to combine the compound or reagent of Inglese with the microarrays of MacBeath in an attempt to achieve the claimed protein chip.

MacBeath in view of Inglese fail to provide any derivative basis for the claimed invention and, additionally, there would have been no logical reason for one of skill in the art to combine such references. Accordingly, no basis of *prima facie* obviousness of the claimed invention is presented by such cited references.

As MacBeath in view of Inglese does not provide any logical basis for the protein chip recited in claim 1, or claims 4-6 dependent therefrom, MacBeath in view of Inglese does not render the claimed invention obvious. Accordingly, withdrawal of the rejection of claims 1-6 under 35 U.S.C. § 103 (a) as being obvious over MacBeath in view of Inglese is respectfully requested.

CONCLUSION

Based on the foregoing, all of applicants' pending claims 1, 4-7, and 9 are patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the January 21, 2009 Office Action without extension was set at three months, or April 21, 2009. Applicants hereby request a 1 month extension of time under 37 CFR § 1.136 to extend the deadline for response to May 21, 2009. Payment of the extension fee of \$65.00 specified in 37 C.F.R. § 1.17(a)(1), as applicable to small entity, is being made by on-line

credit card authorization at the time of EFS submission of this Response. Should any additional fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

Date: May 21, 2009

/steven j. hultquist/
Steven J. Hultquist
Reg. No. 28,021
Attorney for Applicants

Date: May 21, 2009

/kelly k. reynolds/
Kelly K. Reynolds
Reg. No. 51,154
Attorney for Applicants

INTELLECTUAL PROPERTY/
TECHNOLOGY LAW
Phone: (919) 419-9350
Fax: (919) 419-9354
Attorney File No.: 4240-123

Exhibit A – Bradford et al. [7 pgs.]

<p>The USPTO is hereby authorized to charge any deficiency or credit any overpayment of fees properly payable for this document to Deposit Account No. 08-3284</p>

EXHIBIT A

A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding

MARION M. BRADFORD

*Reproduction Research Laboratories, Department of Biochemistry,
University of Georgia, Athens, Georgia 30602*

Received September 11, 1975; accepted January 29, 1976

A protein determination method which involves the binding of Coomassie Brilliant Blue G-250 to protein is described. The binding of the dye to protein causes a shift in the absorption maximum of the dye from 365 to 595 nm. and it is the increase in absorption at 595 nm which is monitored. This assay is very reproducible and rapid with the dye binding process virtually complete in approximately 2 min with good color stability for 1 hr. There is little or no interference from cations such as sodium or potassium nor from carbohydrates such as sucrose. A small amount of color is developed in the presence of strongly alkaline buffering agents, but the assay may be run accurately by the use of proper buffer controls. The only components found to give excessive interfering color in the assay are relatively large amounts of detergents such as sodium dodecyl sulfate, Triton X-100, and commercial glassware detergents. Interference by small amounts of detergent may be eliminated by the use of proper controls.

Laboratory practice in protein purification often requires a rapid and sensitive method for the quantitation of protein. Methods presently available partially fulfill the requirement for this type of quantitation. The standard Lowry procedure (1) is subject to interference by compounds such as potassium ion (2), magnesium ion (3), EDTA (4), Tris (3) thiol reagents (2), and carbohydrates (5). The relatively insensitive biuret reaction (6) is subject to interference by Tris (7), ammonia (8), and glycerol (9). Even the modified procedure for eliminating problems with the Lowry and biuret assays (10,11) present problems since more complications and time are involved in the modified procedures. The dye binding techniques in the literature are for the most part insensitive assays involving the binding of Orange *G* to protein (12-16). The exception to this rule is the Amidoschwarz 10-B binding assay (17). This procedure, too, has its drawbacks since the precipitation of the protein by trichloroacetic acid followed by filtration on Millipore membranes is required.

The protein assay herein described eliminates most of the problems involved in the procedures described above, and is easily utilized for

processing large numbers of samples, as well as adaptable to automation. It is based on the observation that Coomassie Brilliant Blue G-250 exists in two different color forms, red and blue (18). The red form is converted to the blue form upon binding of the dye to protein (18). The protein-dye complex has a high extinction coefficient thus leading to great sensitivity in measurement of the protein. The binding of the dye to protein is a very rapid process (approximately 2 min), and the protein-dye complex remains dispersed in solution for a relatively long time (approximately 1 hr), thus making the procedure very rapid and yet not requiring critical timing for the assay.

MATERIALS AND METHODS

Reagents. Coomassie Brilliant Blue G-250 was obtained from Sigma, and used as supplied. 2-Mercaptoethanol was obtained from Sigma. Triton X-100 was obtained from Schwartz/Mann. Sodium dodecyl sulfate was obtained from BDH Chemicals Ltd., Poole, England. Hemosol was obtained from Scientific Products. All other reagents were of analytical grade or the best grade available.

Protein preparation. Bovine serum albumin (2x crystallized), chymotrypsinogen A, and cytochrome *c* (horse heart) were obtained from Schwartz/Mann. Hemoglobin and human serum albumin were obtained from Nutritional Biochemicals Corporation. Protein solutions were prepared in 0.15 M NaCl. Concentrations were determined for bovine serum albumin, human serum albumin, chymotrypsinogen A, and cytochrome *c* spectrophotometrically in a Bausch and Lomb Spectronic 200 uv spectrophotometer based on $\epsilon_{280}^{1\%} = 6.6$ (19,20), 5.3 (19,21), 20 (19,22) and 17.1 (23,24) respectively. Hemoglobin solutions were prepared gravimetrically.

Preparation of protein reagent. Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 ml 95% ethanol. To this solution 100 ml 85% (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 liter. Final concentrations in the reagent were 0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid.

Protein assay (standard method). Protein solution containing 10 to 100 μg protein in a volume up to 0.1 ml was pipetted into 12 x 100 mm test tubes. The volume in the test tube was adjusted to 0.1 ml with appropriate buffer. Five milliliters of protein reagent was added to the test tube and the contents mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1 hr in 3 ml cuvettes against a reagent blank prepared from 0.1 ml of the appropriate buffer and 5 ml of protein reagent. The weight of protein was plotted against the corresponding absorbance resulting in a standard curve used to determine the protein in unknown samples.

Microprotein assay. Protein solution containing 1 to 10 μg protein in a volume up to 0.1 ml was pipetted into 12 x 100 mm test tubes. The volume of the test tubes was adjusted to 0.1 ml with the appropriate buffer. One milliliter of protein reagent was added to the test tube and the contents mixed as in the standard method. Absorbance at 595 nm was measured as in the standard method except in 1 ml cuvettes against a reagent blank prepared from 0.1 ml of the appropriate buffer and 1 ml of protein reagent. Standard curves were prepared and used as in the standard method.

RESULTS

Reproducibility, sensitivity, and linearity of the assay. Triplicate standard assays of bovine serum albumin as a standard result in a highly reproducible response pattern. Statistical analysis gives a standard deviation of 1.2% of mean value for the assay. There is extreme sensitivity in the assay with 25 μg sample giving an absorbance change of 0.275 OD units. This corresponds to 5 μg protein/ml in the final assay volume. There is a slight nonlinearity in the response pattern. The source of the nonlinearity is in the reagent itself since there is an overlap in the spectrum of the two different color forms of the dye. The background value for the reagent is continually decreasing as more dye is bound to protein. This presents no real problem since the degree of curvature is only slight. If the assay is run with a set of standards and unknowns measured against the response curve of the standards instead of calculated by Beer's Law, there is no difficulty in obtaining satisfactory results.

Accuracy of the assay. Figure 1 shows the results of various proteins assayed in the system as to individual responses. There is a scattering of points around the line drawn in the graph. The scattering is believed to be a multifaceted function composed of difficulties in determining the exact amount of protein present in a given sample due to variation of extinction coefficients in the literature, the methods used to determine the exact amount of protein used in measuring extinction coefficients, and some degree of variation in the efficiency of dye binding to various proteins. Figure 2 shows the response pattern obtained from Lowry (1) assays of the same proteins. The degree of scatter in protein response to Lowry (1) assay is similar to that shown for the dye-binding assay presented here. The sensitivity of the Lowry (1) method is an absorbance of 0.110 OD units for the 25 μg standard corresponding to 8 μg protein/ml of final assay volume. By calculation, then, the dye binding assay is approximately four times more sensitive than the Lowry (1) assay. The degree of scatter around the Lowry (1) assay plot also points to the difficulty in establishing a quantitative value for a protein in standard solutions.

Stability of the protein-dye complex color. Figure 3 shows the rate of

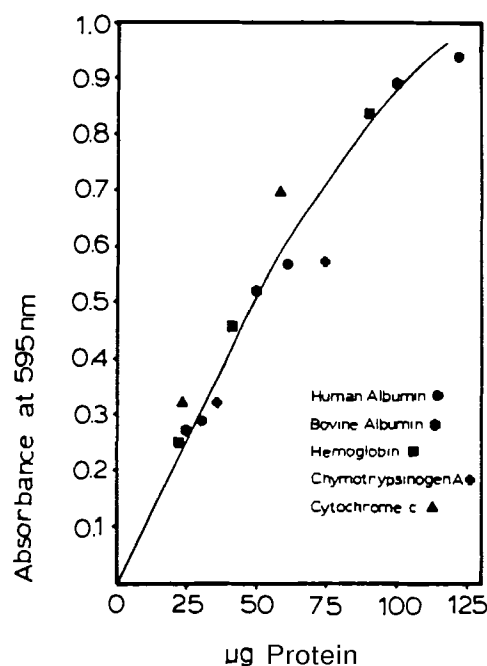


FIG. 1. Protein dye binding response pattern for various proteins.

formation of protein-dye complex in the assay system and the stability of the color complex. The absorbance was monitored at 7.5 sec intervals for 2 min and then at 1 min intervals for a period of 1 hr. As seen from the graph, the color development is essentially complete at 2 min, and remains stable plus or minus 4% for a period of 1 hr. Since the protein-dye complex has a tendency to aggregate with time, there is a decrease in color after this period of time simply by the physical removal of the pretein-dye complex from solution. If very precise determinations are required, investigators should take precaution to read the absorbance of

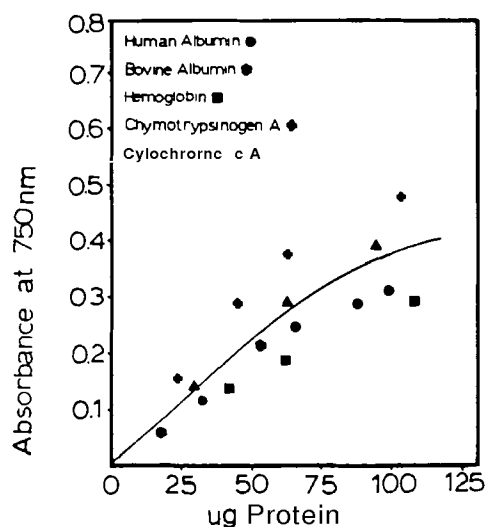


FIG. 2. Lowry (1) response pattern for various proteins.

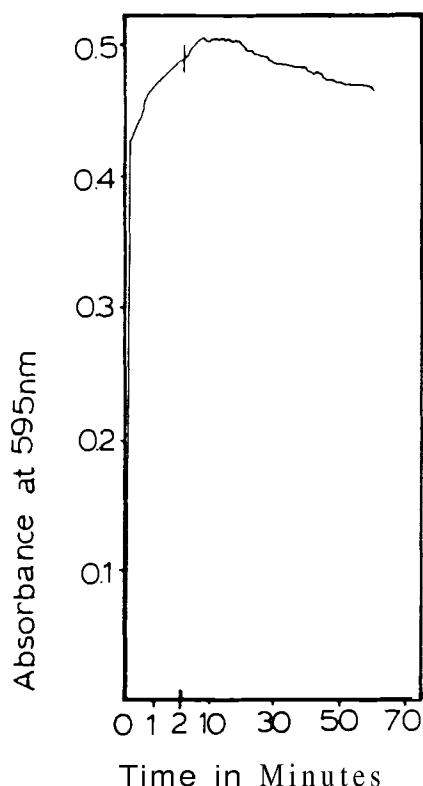


FIG. 3. Protein-dye complex formation rate and color stability.

samples during one of the flatter portions of the color stability curve between 5 and 20 min after reagent addition. This still gives ample time to read a relatively large number of samples.

Microassay system sensitivity. When bovine serum albumin is used as the standard in the micro assay system the degree of nonlinearity is similar to that found in the standard assay. There is a loss in protein-dye complex response as compared with the standard assay, i.e., 5 μg protein/ml gives an absorbance change of 0.1 vs 0.27 in the standard assay. Perhaps this results from increased dilution of the protein reagent.

Interference by nonprotein components. As indicated earlier, there is some interference in the assay system by strongly alkaline buffering agents. This may be overcome by running the appropriate buffer controls and subtracting the value for the control either mathematically or spectrophotometrically. A wide spectrum of components was tested for effects on the protein dye binding assay (Table 1). A lack of effect on the assay by magnesium chloride, potassium chloride, sodium chloride, ethanol, and ammonium sulfate was observed. The small effects due to Tris, acetic acid, 2-mercaptoethanol, sucrose, glycerol, EDTA, and trace quantities of the detergents, Triton X-100, sodium dodecyl sulfate, and Hemosol, can be easily eliminated by running the proper buffer control with the assay. However, the presence of large quantities of the detergents present abnormalities too great to overcome.

TABLE 1
EFFECT OF VARIOUS LABORATORY REAGENTS ON COOMASSIE BRILLIANT
BLUE-G-250-PROTEIN COMPLEX ASSAY^a

Substance	Change in OD 595	(μ g) Equivalent BSA
1 M KCl	0.000	0.00
5 M NaCl	0.000	0.00
1 M MgCl_2	0.000	0.00
2 M Tris	0.026	2.34
0.1 M EDTA	0.004	0.36
1 M $(\text{NH}_4)_2\text{SO}_4$	0.000	0.00
99% Glycerol	0.012	1.08
1 M 2-Mercaptoethanol	0.004	0.36
1 M Sucrose	0.013	1.17
95% Ethanol	0.000	0.00
Acetone	0.069	6.21
5% Phenol	0.046	4.14
0.1% Triton X-100	0.013	1.17
1% Triton X-100	0.590	53.10
0.1% Sodium dodecyl sulfate	0.011	0.99
1% Sodium dodecyl sulfate	0.495	44.55
0.1% Hemosol	0.004	0.36
1% Hemoml	0.108	9.72

^a The above values were obtained when 0.1 ml of each substance was assayed in the standard assay.

A difficulty observed in performing the assay is the tendency of the protein-dye complex in solution to bind to cuvettes. This results in a blue colored cuvette. The amount of binding is negligible as far as assay readings are concerned, i.e., less than 1% error, as indicated by the standard deviation of triplicate assays in the reproducibility section. The blueness of the cuvettes after assay does present problems in other uses of the cuvettes so the following directions for cleaning the blue complex from cuvettes is included:

Method 1: Rinse cuvettes with concentrated glassware detergent, followed by water and acetone. (Gives immediate removal.)

Method 2: Soak cuvettes in 0.1 M HCL. (Removes complex in a few hours.)

The binding of the protein-dye complex has been observed only with quartz cuvettes and may be eliminated by using either glass or plastic cuvettes.

ACKNOWLEDGMENTS

This research was supported by Contract No. I-HD-9-2103 with the Center for Population Research, NICHD and by Ford Foundation Grant No. 680-0805A.

REFERENCES

1. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275.
2. Vallejo, C. G., and Lagunas, R. (1970) *Anal. Biochem.* **36**, 207-212.
3. Kuno, H., and Kihara, H. K. (1967) *Nature (London)* **215**, 974-975.
4. Neurath, A. R. (1966) *Experientia* **22**, 290.
5. Lo, C., and Stelson, H. (1972) *Anal. Biochem.* **45**, 331-336.
6. Mokrasch, L. C., and McGilvery, R. W. (1956) *J. Biol. Chem.* **221**, 909-917.
7. Robson, R. M., Goll, D. E., and Temple, M. J. (1968) *Anal. Biochem.* **24**, 339-341.
8. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) *J. Biol. Chem.* **177**, 751-766.
9. Zishka, M. D., and Nishimura, J. S. (1970) *Anal. Biochem.* **34**, 291-297.
10. Bennett, T. P. (1967) *Nature (London)* **213**, 1131-1132.
11. Grassmann, W., and Hannig, K. (1950) *Naturwissenschaft* **37**, 496-497.
12. Kaul, A. K., Dhar, R. D., and Raghiviah, P. (1970) *J. Food Sci. Technol.* **7**, 11-16.
13. Colenbrander, V. F., and Martin, T. G. (1971) *J. Dairy Sci.* **54**, 531-533.
14. Ashworth, U. S. (1971) *J. Food Sci.* **36**, 509-510.
15. Swaminathan, K., Sud, K. C., and Kishore, H. (1973) *Indian J. Exp. Biol.* **11**, 63-64.
16. Sherbon, J. W. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 1338-1341.
17. Schaffner, W., and Weissmann, C. (1973) *Anal. Biochem.* **56**, 502-514.
18. Reisner, A. H., Nemes, P., and Bucholtz, C. (1975) *Anal. Biochem.* **64**, 509-516.
19. Kirschenbaum, D. M. (1970) in *Handbook of Biochemistry, Selected Data for Molecular Biology* (Sober, H., ed.), 2nd ed., pp. C-71-C-98, Chemical Rubber Company, Cleveland, Ohio.
20. Tanford, C., and Roberts, G. L. (1952) *J. Amer. Chem. Soc.* **74**, 2509-2515.
21. Wetlaufer, D. B. (1962) *Advan. Prot. Chem.* **17**, 378-380.
22. Guy, O., Gratecos, D., Rovey, M., and Desnuelle, P. (1966) *Biochim. Biophys. Acta* **115**, 404-422.
23. Kirschenbaum, D. M. (1973) *Anal. Biochem.* **55**, 166-192.
24. Mayer, M. M., and Miller, J. A. (1970) *Anal. Biochem.* **36**, 91-100.